Polyacetylenes from Sardinian *Oenanthe fistulosa*: A Molecular Clue to *risus sardonicus*

Giovanni Appendino,*[,†,‡] Federica Pollastro,[†,‡] Luisella Verotta,[§] Mauro Ballero,[‡] Adriana Romano,[⊥] Paulina Wyrembek,[¶] Katarzyna Szczuraszek,[¶] Jerzy W. Mozrzymas,*[,¶] and Orazio Taglialatela-Scafati*[,⊥]

Dipartimento di Scienze Chimiche, Alimentari, Farmaceutiche e Farmacologiche, Università del Piemonte Orientale, Viale Ferrucci 33, 28100 Novara, Italy, Consorzio per lo Studio dei Metaboliti Secondari (COSMESE), Viale S. Ignazio 13, 09123 Cagliari, Italy, Dipartimento di Chimica Organica e Industriale, Università di Milano, Via Venezian 21, 20133 Milano, Italy, Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli Federico II, Via Montesano 49, 80131 Napoli, Italy, and Laboratory of Neuroscience, Department of Biophysics, Wroclaw Medical University, ul. Chalubinskiego 3, 50-368 Wroclaw, Poland

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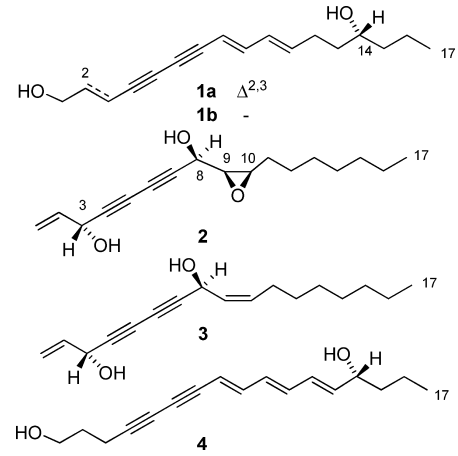
An investigation of *Oenanthe fistulosa* from Sardinia afforded oenanthotoxin (**1a**) and dihydrooenanthotoxin (**1b**) from the roots and the diacetylenic epoxydiol **2** from the seeds. The absolute configuration of **1a** and **1b** was established as *R* by the modified Mosher's method, and the structure of **2** by chemical correlation with (+)-(3*R*,8*S*)-falcarindiol. Oenanthotoxin (**1a**) and dihydrooenanthotoxin (**1b**) were found to potently block GABAergic responses, providing a molecular rationale for the symptoms of poisoning from water-dropwort (*Oenanthe crocata*) and related plants. These observations bear relevance for a series of historical and ethnopharmacological observations on the identification of the Sardonic herb and the molecular details of the facial muscular contraction caused by its ingestion (*risus sardonicus*).

Plants from the genus *Oenanthe* (Umbelliferae family) are among the most poisonous species of the European flora.[1] They bear a general resemblance to parsnip and carrot and are still a cause of often fatal human poisonings.[1] Sardinian *Oenanthe* species have a special ethnopharmacological relevance, being considered the most likely candidate for the (in)famous sardonic herb, a neurotoxic plant used in pre-Roman Sardinia for the ritual killing of elderly people.[2] According to the ancient historians, elderly people unable to support themselves were intoxicated with the sardonic herb and then killed by dropping from a high rock or by beating to death.[2] The facial muscular contraction induced by the sardonic herb mimicked a smile, and the expression *risus sardonicus* (sardonic smile) to indicate a sinister smile is well documented in the Latin and Greek literature and in most modern European languages. It even found its way into the mainstream medical lingo as the hallmark of lockjaw (*trismus*), the spasm of the muscles of mastication.[3]

The poisonous constituents of *Oenanthe* species are a series of polyacetylenic alcohols exemplified by oenanthotoxin (**1a**).[4] Surprisingly, the absolute configuration at the stereogenic carbon (C-14) of oenanthotoxin is still unknown, and little information on the molecular details of its neurotoxic activity has been reported. As part of a study on poisonous Sardinian plants,[5] we have investigated *O. fistulosa* L. (tubular water-dropwort), a species that, despite its very broad distribution in Europe, had so far been overlooked in terms of phytochemical studies. Large amounts of the diacetylene epoxydiol **2** were obtained from the seeds, while the roots gave, along with the widespread acetylene falcarindiol (**3**), oenanthotoxin (**1a**) and dihydroenanthotoxin (**1b**). The absolute configuration of **1a** and **1b** was clarified by application of the modified Mosher method, while the activity of oenanthotoxin was profiled against GABA receptors, a target involved in the action of many convulsivant toxins of plant origin.[6]

An acetone extract from the underground parts of *O. fistulosa* was fractionated by gravity column chromatography to afford large amounts (ca. 0.80% of dried plant material) of (+)-(3*R*, 8*S*)-falcarindiol (**3**)[7] and a mixture of oenanthotoxin (**1a**) and its dihydroderivative (**1b**) (0.070%).[4] Compounds **1a** and **1b** were easily separated by preparative HPLC and identified by comparison with authentic samples of (+)-oenanthotoxin and (+)-dihydrooenanthotoxin obtained, in over 10-fold higher yield, from the roots of *O. crocata*, a more common species in Sardinia (see Experimental Section). A complete assignment of the ^{1}H NMR resonances of both **1a** and **1b** is reported in the Experimental Section.



Oenanthotoxin (**1a**) is isomeric with cicutoxin (**4**) from water hemlock (*Cicuta virosa* L.), with the only difference between these C-17 acetylenic toxins being the position of a single double bond, located next to the secondary oxymethine in cicutoxin and adjacent to the primary hydroxyl in oenanthotoxin.[4b] The absolute configuration at C-14 of cicutoxin was established as *R* by application of a CD exciton chirality method on its *p*-methoxybenzoate, taking advantage of the allylic nature of the stereogenic center, and was confirmed by application of the Mosher method.[8] Since the stereogenic center of oenanthotoxin is not allylic, the Mosher method[9] was used to assign its absolute configuration.

To achieve this aim, two aliquots of oenanthotoxin (**1a**) were dissolved in dry pyridine and allowed to react overnight with DMAP and (*R*)- or (*S*)-MTPA chloride, respectively, affording the (*S*)- and (*R*)-MTPA diesters **5a** and **5b**, respectively. Analysis of the $\Delta\delta(S-R)$ values of the protons neighboring the oxygenated methine according

* To whom correspondence should be addressed. Tel: +39 0321 375744 (G.A.); +48 71 7841550 (J.W.M.); +39 081 678509 (O.T.-S.). Fax: +39 0321 375621 (G.A.); +48 71 784 1399 (J.W.M.); +39 081 678552 (O.T.-S.). E-mail: appendino@pharm.unipmn.it (G.A.); mozrzy@biofiz.am.wroc.pl (J.W.M.); scatagli@unina.it (O.T.S.).
† Università del Piemonte Orientale.
‡ Consorzio per lo Studio dei Metaboliti Secondari.
§ Università di Milano.
⊥ Università di Napoli Federico II.
¶ Wroclaw Medical University.

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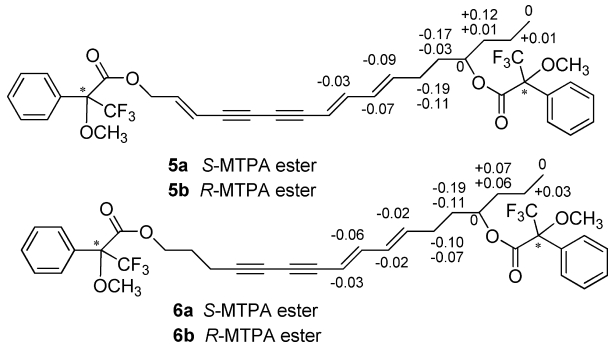

Figure 1. Application of the modified Mosher's method for secondary alcohols on the MTPA esters of oenanthotoxin (**5a** and **5b**) and dihydrooenanthotoxin (**6a** and **6b**). $\Delta\delta(\delta_S-\delta_R)$ values are given in ppm.

to the Mosher model[9] (Figure 1) allowed the assignment of the *R* configuration at C-14 of **1a**. The same methodology was applied to dihydrooenanthotoxin (**1b**), which, by analysis of the $\Delta\delta(S-R)$ values between its (*S*)- and (*R*)-MTPA diesters **6a** and **6b** (Figure 1), turned out to have the same *R* configuration at C-14. In both cases, concomitant MTPA esterification at C-1 did not exert any influence on the anisotropic effect of the phenyl group of the MTPA moiety bound to the C-14, located 13 carbons away from the primary hydroxyl.

The seeds of *O. fistulosa* were totally devoid of oenanthotoxin-like polyacetylenes and contained rather large amounts of the diacetylenic epoxydiol **2**. The structure elucidation of this compound ($C_{17}H_{24}O_3$, HRMS) was helped greatly by comparison with falcarindiol (**3**),[7] since the two compounds showed similar spectroscopic features.[7] Thus, **2** differed from **3** only in the epoxidation of the disubstituted double bond, as evident from the replacement of the two mutually coupled olefin protons of **3** with two oxymethine signals [δ 3.06 (dd, $J = 7.1$, 4.0 Hz, H-9) and 2.80 (dt, $J = 5.9$, 4.0 Hz, H-10]. Corresponding changes were observed in the ^{13}C NMR spectrum (upfield shift of the C-9 and C-10 methines, now resonating at δ 57.9 and 57.4, respectively). Full assignment of ^{1}H and ^{13}C NMR resonances of **2** was achieved through analysis of 2D NMR (COSY, HSQC, and gHMBC) data, which confirmed that **2** is an epoxidized derivative of falcarindiol. To further confirm this assignment and establish the configuration of the epoxide ring, the reaction of (+)-(3*R*,8*S*)-falcarindiol (**3**)[7] with peracids was investigated. Enines can be chemoselectively epoxidized, and treatment of falcarindiol with *meta*-chloroperoxybenzoic acid (MCPBA) under controlled conditions (monitoring of the conversion by ^{1}H NMR analysis, see Experimental Section) gave the epoxide **2** in satisfactory (66%) yield and as the only reaction product. This was identical in terms of both spectroscopic properties and optical rotation to the natural product isolated from *O. fistulosa*. Prolonged reaction times or the addition of $NaHCO_3$ to buffer the acidity of the medium led to extensive degradation of the reaction product. The epoxidation of allylic alcohols is a *syn* addition, and the peroxy acid is directed by hydrogen bonding to the allylic hydroxyl, which, in the case of secondary alcohols, reacts in the conformation that minimizes allylic strain ($A^{(1,3)}$-strain) between the alkyl group bound to the oxymethine and the distal substituent of the adjacent double bond (C-7 and C-11, respectively, in the case of the epoxidation of **3**).[10] Taking into account that the stereogenic double bond of falcarindiol has a *cis* configuration, these considerations lead to a 9*S*,10*R* configuration for the epoxide ring of **2**.[11] A compound with the planar structure of **2** and spectroscopic features compatible with those of **2** was described from *Panax quinquefolium*.[12] However, the optical rotation was different [+ 107 (MeOH) for the epoxydiol from *O. fistulosa* and +87 (MeOH) for that from *P. quinquefolium*], and in the absence of an authentic sample, the identity of the two compounds could not be established.

Polyacetylene neurotoxins are believed to act like biological analogues of the sesquiterpene lactone picrotoxin, binding to the convulsant site of $GABA_A$ receptors and causing death by inducing convulsions and respiratory paralysis.[13] In addition, oenanthotoxin has also been reported to dramatically affect cationic (calcium and sodium) currents in excitable membranes.[14] We found that both oenanthotoxin and dihydrooenanthotoxin could potently block the GABAergic responses in neuronal cell cultures, with an EC_{50} value in the low micromolar range for both compounds. Currents elicited by exogenous GABA were recorded in the whole-cell configuration by rapid application of 3 μM GABA at the membrane voltage of -40 mV, and a typical GABA-evoked response, recorded in these conditions, is presented in Figure 2 (left). The effect of **1a** and **1b** on current amplitude was assessed by dividing the current amplitude measured in the presence of this drug by the amplitude value in control conditions measured from the same cell. Oenanthotoxin (**1a**) clearly inhibited the GABAergic responses in a dose-dependent manner (Figure 2A). A tentative fitting of the logistic equation ($y = 1/(1 + (c/Ec_{50})h)$) to the amplitude dose-dependence for oenanthotoxin shown in Figure 2B yielded a EC_{50} value of 1.39 μM ($h = 0.59$). Dihydrooenanthotoxin (**1b**) exhibited a similar inhibitory activity on currents elicited by 3 μM GABA, but was slightly more potent ($EC_{50} = 0.835$ μM, $h = 0.58$). The effect of both test compounds was reversible, although at least 10 min of wash was required to restore the control responses. Compound **1b** can be viewed both as 2,3-dihydrooenanthotoxin and as 12,13-dihydrocicutoxin (cf. **1a** and **4**), showing that, in polyacetylenic toxins, the presence of allylic double bonds is redundant for activity and that the conjugated system required for the activity is shorter than previously postulated,[13] encompassing four, and not five, unsaturations.

Unlike other plant toxins, the convulsant polyacetylenes from water dropwort and related plant do not evoke unpleasant taste (bitter) or chemesthetic (burning) sensations, and the roots of *O. crocata*, an exceedingly poisonous plant, have a paradoxical sweetish and pleasant taste and odor.[15] The large concentration of falcarindiol, a bitter compound,[16] and the lower contents of polyacetylene toxins in *O. fistulosa* when compared to *O. crocata* could presumably underlie the observation that the former species has not yet been associated with human or animal poisoning. The name *Oenanthe* signifies "wine flower", because the plant produces a state of stupefaction similar to drunkenness.[1,2] This, as well as locked jaws (*risus sardonicus*), has been documented in human poisoning from *O. crocata*,[1,2] and there is little doubt that *herba sardonica* of the ancient medical literature should be identified with *O. crocata*,[2] a plant that, within the Mediterranean area, is common only in Sardinia.[2] The results of our investigation provide a further rationale for this identification, proposing a molecular mechanism for the *risus sardonicus* described by the ancient authors.

Neurotoxins have emerged as powerful neurochemical tools,[17,18] and the scarce attention given so far to the C-17 neurotoxic acetylenes can best be explained by their chemical instability, especially in the highly purified form required for biological studies. Thus, oenanthotoxin (**1a**) and dihydrooenanthotoxin (**1b**), just like many other polyacetylenes, easily turned into highly colored and insoluble polymeric materials upon storage, even at low temperature (−18 °C). To overcome this problem, we have developed a flash chromatography protocol to separate oenanthotoxin (**1a**) and dihydroenanthotoxin (**1b**) from a crude extract of *O. crocata* and found that these compounds, while being highly unstable in the solid state, can be conveniently stored for at least 6 months in benzene or DMSO solution at 4 °C (see Experimental Section). This might foster interest in this fascinating class of compounds, with its unique capacity of dramatically perturbing calcium and sodium ionic currents in excitable membranes[14] and specifically interacting with ligand-gated ion channels like the $GABA_A$ receptor.

Experimental Section

General Experimental Procedures. Optical rotations ($CHCl_3$) were measured at 589 nm on a Perkin-Elmer 192 polarimeter equipped with a sodium lamp ($\lambda = 589$ nm) and a 10 cm microcell. ^{1}H (500 MHz)

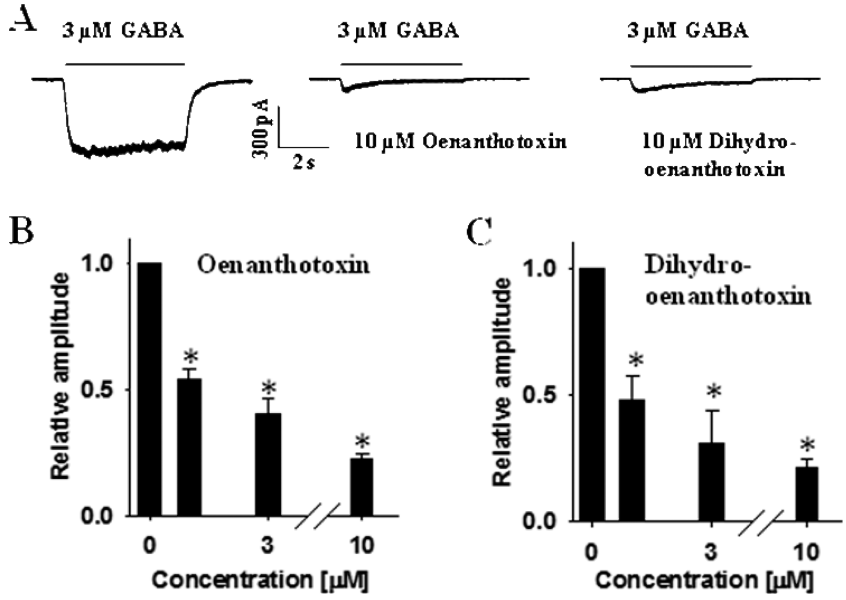

Figure 2. Oenanthotoxin (**1a**) and dihydrooenanthotoxin (**1b**) inhibit GABA-evoked responses in a dose-dependent manner. (A) Typical current traces evoked by rapid application of 3 μM GABA at −40 mV in control conditions (left), in the presence of 10 μM **1a** (middle), and in the presence of 10 μM **1b** (right). (B) Dose-dependent effect of **1a** on GABA-evoked current amplitude relative value. (C) Dose-dependent effect of **1b**. Each data point in B and C was calculated from at least 3 values. Asterisks above the bars indicate statistically significant difference.

and ^{13}C (125 MHz) NMR spectra were measured on a Varian INOVA spectrometer. Chemical shifts were referenced to the residual solvent signal ($CDCl_3$: δ_H 7.26, δ_C 77.0). Homonuclear ^1H connectivities were determined by the COSY experiment. One-bond heteronuclear $^1H-^{13}C$ connectivities were determined with the HSQC experiment. Two- and three-bond $^1H-^{13}C$ connectivities were determined by HMBC experiments optimized for a $^{2,3}J$ of 7 Hz. Through-space ^1H connectivities were evidenced using a ROESY experiment with a mixing time of 500 ms. Two- and three-bond $^1H-^{13}C$ connectivities were determined by gradient 2D HMBC experiments optimized for a $^{2,3}J$ of 9 Hz. Low- and high-resolution EIMS spectra (70 eV) were performed on a VG Prospec (Fisons) mass spectrometer. ESIMS spectra were performed on a LCQ Finnigan MAT mass spectrometer. Silica gel 60 (70−230 mesh) was used for gravity column chromatography. Reactions were monitored by TLC on Merck 60 F_{254} (0.25 mm) plates, which were visualized by UV inspection and/or staining with 5% H_2SO_4 in ethanol and heating. Organic phases were dried with Na_2SO_4 before evaporation.

Plant Material. *Oenanthe fistulosa* was collected near San Basilio (CA, Sardinia, Italy) on September 14, 2007. The plant was identified by M.B., and a voucher specimen (171007) is held at the University of Cagliari. *O. crocata* was collected near Bonacardo (Abbasanta, NU, Italy) in May 8, 2007, and a voucher specimen (OCR080506BO) is held at the University of Cagliari.

Extraction and Isolation. (A) Powdered, dried underground parts of *O. fistulosa* (608 g) were extracted with acetone (2 × 2 L) at room temperature, affording 7.94 g of a dark oil, which was fractionated by gravity column chromatography on silica gel (*n*-hexane−EtOAc, 9:1 to 6:4, as solvent gradient system). Fractions eluted with *n*-hexane−EtOAc (9:1) were further purified by HPLC (*n*-hexane−EtOAc, 85:15) to yield falcarindiol (**3**, 344 mg). Fractions eluted with *n*-hexane−EtOAc (6:4) were further purified by HPLC (*n*-hexane−EtOAc, 65:35) to yield oenathotoxin (**1a**, 68 mg) and dihydrooenanthotoxin (**1b**, 67 mg). (B) Powdered, dried seeds of *O. fistulosa* (379 g) were extracted with acetone (2 × 2 L) at rt, affording 21.3 g of a yellowish oil, which was fractionated by gravity column chromatography on silica gel (*n*-hexane−EtOAc, 9:1 to 6:4, as solvent gradient system). Fractions eluted with *n*-hexane−EtOAc (7:3) were further purified by HPLC (*n*-hexane−EtOAc, 55:45) to yield epoxyfalcarindiol (**2**, 432 mg). (C) Dried underground parts of *O. crocata* (95 g) were extracted with acetone (2 × 1 L) to afford 3.1 g of a brownish oil, which was purified with the Biotage SP1 system with a silica gel (10 g) FLASH 12+M column and a *n*-hexane−EtOAc gradient of increasing polarities (flow rate 9 mL/min, UV detector 316 nm; fractionation mode: volume 9 mL/fraction). Fractions 29−31 and 34−36 (*n*-hexane−EtOAc, 6:4) afforded about 88 mg of oenanthotoxin (**1a**) and 89 mg of dihydrooenanthotoxin (**1b**), respectively. These compounds were obtained as colorless foams and could not be stored in this form. However, solutions in hydrocarbon solvents (benzene, toluene) or DMSO could be stored for at least 6 months at 4 °C without any apparent (^1H NMR analysis) degradation or development of a reddish color.

Oenanthotoxin (1a): amorphous foam; $[\alpha]_D^{25}$ +34 (*c* 0.5, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 6.71 (1H, dd, J = 15.5, 11.0 Hz, H-9), 6.39 (1H, dt, J = 15.0, 5.0 Hz, H-2); 6.12 (1H, dd, J = 15.0, 11.0 Hz, H-10), 5.87 (1H, overlapped H-3), 5.86 (1H, overlapped, H-11); 5.57 (1H, d, J = 15.5 Hz, H-8), 4.25 (2H, d, J = 5.0 Hz H_2-1), 3.60 (1H, m, H-14), 2.30 (1H, m, H-12a), 2.20 (1H, m, H-12b), 1.78 (1H, m, H-13a), 1.50 (1H, overlapped, H-13b), 1.49 (1H, overlapped, H-15a), 1.40 (1H, m, H-15b), 1.30 (2H, m, H_2-16), 0.92 (3H, d, J = 7.3, Hz H_3-17); ESIMS (positive ion) *m*/*z* 281 $[M + Na]^+$.

Dihydrooenanthotoxin (1b): amorphous foam; ^1H NMR (500 MHz, $CDCl_3$) δ 6.65 (1H, dd, J = 15.5, 10.6 Hz, H-9), 6.12 (1H, dd, J = 15.0, 11.0 Hz, H-10), 5.84 (1H, dt, J = 15.0, 5.0 Hz, H-11), 5.50 (1H, d, J = 15.5 Hz, H-8), 3.77 (2H, t, J = 5.0 Hz, H_2-1), 3.60 (1H, m, H-14), 2.45 (2H, t, J = 5.0 Hz, H_2-3), 2.30 (1H, m, H-12a), 2.21 (1H, m, H-12b), 1.79 (2H, t, J = 5.0 Hz, H_2-2), 1.75 (1H, m, H-13a), 1.50 (1H, overlapped, H-13b), 1.49 (1H, overlapped, H-15a), 1.40 (1H, m, H-13b), 1.30 (2H, m, H_2-16), 0.93 (3H, d, J = 7.3 Hz, H_3-17); ESIMS (positive ion): *m*/*z* 283 $[M + Na]^+$.

9-Epoxyfalcarindiol (2): colorless oil, $[\alpha]_D^{25}$ +107 (*c* 0.9, MeOH); IR (liquid film) ν_{max} 3400, 3120, 2080, 1451, 1293, 1238, 1164, 983 cm^{-1}; ^1H NMR (500 MHz, C_6D_6) δ 5.78 (1H, ddd, J = 17.0, 10.2, 5.45 Hz, H-2), 5.37 (1H, brd, J = 17.0 Hz, H-1a), 5.03 (1H, brd, J = 10.3 Hz, H-1b), 4.67 (1H, d, J = 5.4 Hz, H-3), 4.20 (1H, d, J = 7.1 Hz, H-8), 3.06 (1H, dd, J = 7.1, 4.0 Hz, H-9), 2.80 (1H, dt, J = 5.9, 4.0 Hz, H-10), 1.53 (2H, brm, H-11a,b), 1.45−1.30 (10H, m, H-12a,b + H-13a,b + H-14a,b + H-15a,b + H-16a,b), 1.01 (3H, t, J = 7.1 Hz, H-17); ^{13}C NMR (125 MHz, $CDCl_3$) δ 136.1 (d, C-2), 116.2 (t, C-1), 79.3 (s, C-7), 79.1 (s, C-4), 70.3 (s, C-5), 70.1 (s, C-6), 63.2 (d, C-3), 61.0 (d, C-8), 57.9 (d, C-9), 57.4 (d, C-10), 31.9 (t, C-15), 27.6 (t, C-11), 29.4, 29.3 (t, C-13 and C-14) 26.6 (t, C-12), 22.8 (t, C-16), 14.1 (q, C-17). HREIMS *m*/*z* 276.1737 $[M]^+$ (calcd for $C_{17}H_{24}O_3$, 276.1725).

MTPA Esters of Oenanthotoxin and Dihydrooenanthotoxin. Oenanthotoxin (**1a**, 2.5 mg) was dissolved in 0.5 mL of dry pyridine and treated with (−)-*R*-MTPA chloride (20 μL) and *N*,*N*-dimethylaminopyridine (DMAP, a spatula tip), then maintained at room temperature under stirring overnight. After removal of the solvent, the reaction mixture was purified by HPLC on a SI60 column (eluent *n*-hexane−EtOAc, 9:1), affording the (*S*)-MTPA diester **1c** in a pure state

(3.5 mg, 53% yield). Using (+)-*S*-MTPA chloride, the same procedure afforded the (*R*)-MTPA diester **1d** in the same yield. This procedure was repeated for dihydrooenanthotoxin (**1b**, 2.0 mg) to obtain the (*S*)-MTPA diester **1e** and (*R*)-MTPA diester **1f** in the same yield.

Oenanthotoxin-1-*O*-14-*O*-(S)-MTPA diester (5a): amorphous solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 and 7.35 (MTPA phenyl protons), 6.66 (H-9, dd, J = 15.5, 10.6 Hz), 6.28 (H-2, dt, J = 15.0, 5.0 Hz), 6.09 (H-10, dd, J = 15.0, 10.6 Hz), 5.84 (H-3, overlapped), 5.82 (H-11, overlapped), 5.57 (H-8, d, J = 15.5 Hz), 5.10 (H-14, m), 4.86 (H_2-1, d, J = 5.0 Hz), 3.55 (MTPA OCH_3, s), 2.16 (H-12a, overlapped), 2.12 (H-12b, overlapped), 1.81 (H-13a, m), 1.73 (H-15a, m), 1.65 (H-13b, m), 1.58 (H-15b, m), 1.29 (H_2-16, m), 0.88 (H_3-17, d, J = 7.3 Hz); FABMS (glycerol matrix, positive ions) m/z 691 $[M + H]^+$.

Oenanthotoxin-1-*O*-14-*O*-(R)-MTPA diester (5b): amorphous solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 and 7.53 (MTPA phenyl protons), 6.69 (H-9, dd, J = 15.5, 10.6 Hz), 6.26 (H-2, dt, J = 15.0, 5.0 Hz), 6.16 (H-10, dd, J = 15.0, 10.6 Hz), 5.91 (H-11, overlapped), 5.86 (H-3, overlapped), 5.57 (H-8, d, J = 15.5 Hz), 5.10 (H-14, m), 4.86 (H_2-1, d, J = 5.0 Hz), 3.58 (MTPA OCH_3, s), 2.35 (H-12a, m), 2.23 (H-12b, m), 1.98 (H-13a, m), 1.68 (H-13b, m), 1.61 (H-15a, m), 1.57 (H-15b, m), 1.28 (H_2-16, m), 0.88 (H_3-17, d, J = 7.3 Hz); FABMS (glycerol matrix, positive ions) m/z 691 $[M + H]^+$.

Dihydrooenanthotoxin-1-*O*-14-*O*-(S)-MTPA diester (6a): amorphous solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 and 7.35 (MTPA phenyl protons), 6.64 (H-9, dd, J = 15.5, 10.6 Hz), 6.09 (H-10, dd, J = 15.0, 10.6 Hz), 5.83 (H-11, dt, J = 15.0, 5.0 Hz), 5.45 (H-8, d, J = 15.5 Hz), 5.20 (H-14, m), 4.39 (H_2-1, m), 3.55 (MTPA OCH_3, s), 2.36 (H_2-3, t, J = 6.5 Hz), 2.25 (H-12a, overlapped), 2.21 (H-12b, overlapped), 1.91 (H_2-2, m), 1.75 (H-15a, m), 1.71 (H-13a, m), 1.60 (H-15b, m), 1.56 (H-13b, m), 1.30 (H_2-16, m), 0.90 (H_3-17, d, J = 7.3 Hz); FABMS (glycerol matrix, positive ions) m/z 693 $[M + H]^+$.

Dihydrooenanthotoxin-1-*O*-14-*O*-(R)-MTPA diester (6b): amorphous solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 and 7.53 (MTPA phenyl protons), 6.70 (H-9, dd, J = 15.5, 10.6 Hz), 6.11 (H-10, dd, J = 15.0, 10.6 Hz), 5.85 (H-11, dt, J = 15.0, 5.0 Hz), 5.48 (H-8, d, J = 15.5 Hz), 5.20 (H-14, m), 4.39 (H_2-1, m), 3.58 (MTPA OCH_3, s), 2.36 (H_2-3, overlapped), 2.35 (H-12a, overlapped), 2.28 (H-12b, overlapped), 1.91 (H_2-2, m), 1.90 (H-13a, m), 1.68 (H-15a, overlapped), 1.67 (H-13b, overlapped), 1.54 (H-15b, m), 1.27 (H_2-16, m), 0.90 (H_3-17, d, J = 7.3 Hz); FABMS (glycerol matrix, positive ions) m/z 693 $[M + H]^+$.

Epoxidation of Falcarindiol. To a solution of falcarindiol (85 mg, 0.36 mmol) in dry CH_2Cl_2 (3 mL) was added 85% *m*-chloroperbenzoic acid (MCPBA, 73 mg, 0.36 mmol, 1 molar equiv). The course of the reaction was followed by ^{1}H NMR analysis of a $CDCl_3$ solution containing an equimolecular mixture of falcarindiol and MCPBA. After stirring 2 h at rt, the reaction was worked up by dilution with CH_2Cl_2 and the addition of silica gel (1 g) impregnated with a 5% water solution of $Na_2S_2O_3$. After stirring a few minutes, the slurry was filtered and the filtrate was dried (Na_2SO_4) and evaporated, to afford 60 mg (66%) of 9-epoxyfalcarindiol (**2**), spectroscopically (^{1}H and ^{13}C NMR) identical to the natural product and having $[\alpha]_D^{25}$ +101 (*c* 1.1, MeOH).

Cell Culture for Electrophysiological Recordings. Neuronal cell culture was prepared as previously described in detail by Andjus et al.[19] Briefly, P1−P3-day-old Wistar rats were decapitated. This procedure is in accordance with the regulation of the Polish Animal Welfare Act. Hippocampi were removed, manually sliced, treated with trypsin, mechanically dissociated, centrifuged twice at 40 g, plated in Petri dishes, and cultured. Experiments were performed on cells between 10 and 17 days in culture.

Electrophysiological Recordings. Currents were recorded in the whole-cell configuration of the patch-clamp technique using the Axopatch 200B amplifier (Molecular Instruments, Sunnyvale, CA) at a holding potential (V_h) of −40 mV. The intrapipet solution contained (in mM) CsCl 137, $CaCl_2$ 1, $MgCl_2$ 2, 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′-tetraacetic acid (BAPTA) 11, ATP 2, and HEPES 10 (pH 7.2 with CsOH). The composition of the standard external solution was (in mM) NaCl 137, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, glucose 20, and HEPES 10 (pH 7.2 with NaOH). For the whole-cell recordings, patch pipets had a resistance of 2.5−4.0 MΩ when filled with internal solution. The whole-cell recordings were included in the statistics when the access resistance was below 10 MΩ. The cells in which series resistance showed a clear tendency to increase during recordings were not considered in the analysis. Recordings in the whole-cell mode were started at least 3 min after establishing the whole-cell mode. This time was sufficient to stabilize the recording conditions. The agonist was applied using the RSC-200 multibarrel rapid perfusion system (Bio-Logic, Grenoble, France). With this system, in the whole-cell configuration, the solution exchange occurred within 30−100 ms. Before each control recording, cells were washed with normal external solution for at least 3 min. In studies aiming at assessment of the impact of considered test compounds [oneantotoxin (**1a**), dihydrooneantotoxin (**1b**)] on GABA-elicited responses, these compounds were present both in the washing solution (for at least 3 min before agonist application) and in the agonist-containing saline. For acquisition and data analysis, pClamp 9.2 software was used (Molecular Devices Corporation). To avoid the data scatter due to cell-to-cell variability, the effect of studied drugs was assessed by calculating the relative amplitude values with respect to the controls recorded from the same cell. For the analysis of currents, recorded in the whole-cell configuration, the current signals were low-pass filtered at 3 kHz with a Butterworth filter and sampled at 10 kHz using the analog-to-digital converter Digidata 1322A (Molecular Device Corporation) and stored on a computer hard disk. Data are expressed as mean ± SEM. A paired Student *t* test was used to assess the significance of differences between considered data sets. All experiments were performed at room temperature, 22−24 °C.

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